Prevalence of S.epidermidis and S.aureus and their biofilm ability among Iraqi patients suffering from urinary tract infection

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Abstract

Urinary tract infections (UTI) caused by methicillin resistant staphylococci are a growing problem for many health care institutions especially when it correlates with biofilms formation of these isolates on living and nonliving surfaces. The prevalence of staphylococci from UTI were studied and it was found that S.epidermidis are higher prevalence than S.aureus 55.5% (10 out of 18) and 26.6% (8 out of 30) were methicillin resistant staphylococcus aureus isolates (MRSA) and methicillin resistant staphylococcus epidermidis (MRSE), respectively. Biofilm formation on microtiter plates revealed that MRSE isolates was more efficient in biofilm production than its counterpart MRSA.

Keywords:UTI, Biofilm, MRSA, MRSE.

انتشار العنقوديات الذهبية والعنقوديات البشروية وقدرتها على تكوين الاغشية الحيوية للمرضى العراقيين الذين يعانون من خمج المجاري البولية

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الخلاصة

يعد التهابات المجاري البولية (UTI) التي تسببها المكورات العنقودية المقاومة للميثيسيلين اهم مشكلة متنامية بالنسبة للعديد من المؤسسات الصحية وخصوصا عندما ترتبط مع قدرة هذه العزلات على انتاج الأغشية الحياتية على السطوح الحيه وغير الحيه. تم دراسة مدى انتشار المكورات العنقودية من الاشخاص المصابين بالالتهاب المجاري البولية اذ وجد أن S.epidermidis ابدت أعلى معدل انتشار من S.aureus ، وان 55.5% (بواقع عشر عزلات من اصل ثمانيه عشر عزله) و 26.6 % (بواقع ثمان عزلات من اصل ثلاثون عزله) كانت مكورات عنقودية ذهبية مقاومة للميثيسيلين MRSA و مكورات عنقودية بشروية مقاومة للميثيسيلين MRSE على التوالي. من جانب اخر فقد اظهرت دراسة انتاج الاغشية الحياتية ان عزلات MRSE كانت اكثر كفاءه من نضيرتها MRSA.

الكلمات المفتاحيه:خمج المجاري البوليه،الاغشيه الحيويه،العنقوديات الذهبيه المقاومه للمثيسلين،العنقوديات البشرويه المقاومه للمثيسلين

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Introduction:

A urinary tract infection (UTI) considered as an immune trouble due to changes in the immune system of the patients and the increased emergence of resistance to antibiotics of prevalent strains [1].

Infections of the urinary tract are the second most common type of infection in the body. UTI's accounted for about 8.3 million doctor visits in the US in 1997[2] and 40% of the nosocomial infections [3]. The genus most associated with UTIs is Staphylococci which normally forms biofilm on implanted medical devices [4].The pathogenicity of S.aureus and S. epidermidis is associated with a number of bacterial virulence factors such as extracellular enzymes [5]. In addition to its ability to form biofilm on surfaces such as indwelling medical devices [6 and 7].

Material and method:

Specimens' collection

One ml of midstream urine was collected in a sterile, wide-mouth container. Unpreserved specimens were cultured within 2hr. of collection or stored in a refrigerator for no more than 24hr. [9].

Isolation of bacteria

A loopful of urine sample was directly inoculated to 5ml 0f brain-heart nfusion(BHI broth), then transferred to plates of Mannitol Salt Agar (MSA) and incubated at 37°C for 24 hr. All colonies from primary cultures were purified by subculture on brain-heart infusion (BHI) agar and then re-inoculated onto MSA and incubated at 37°C for 24 hr. [10]. S.aureus and S.epidermidis were identified Nosocomial infections caused by multiresistant staphylococci are a growing problem for many health care institutions and the basis of this resistance is conferred by an additional penicillin-binding protein (PBP-2a), which is absent in methicillin susceptible strains. From all species of staphylococci, S.epidermidis and S. aureus have the greatest pathogenic potential [8].

The aim of this study is study the prevalence of staphylococci spp. among UTI and to find the difference in terms of biofilm productivity between methicillin resistant Staphylococcus aureus (MRSA) and methicillin resistant Staphylococcus epidermidis (MRSE) isolates from UTI patients.

bygusing biochemical tests including: Gram stain, catalase test, oxidase test, tube coagulase test, manitol salt agar, motility test and API staph system in order to confirm diagnosis [11].

Detection of Methicillin Resistant S. aureus (MRSA) and Methicillin Resistant S. epidermidis MRSE)

Oxacillin disk (1µg) diffusion method was used to detect MRSA and MRSE according to the CLSI guidelines [12]. A 0.5 McFarland standard suspension of the isolate was made and lawn culture done on muller hinton agar plate. Plates were incubated at 37℃ for 18 hr and zone diameters were measured. An inhibition zone diameter of ≤ 10 mm was reported as Methicillin resistant, ≥13 mm was considered as Methicillin sensitive and 11–12 as Methicillin intermediate.

Biofilm assays:

Biofilm assays were performed in 96-well microtiter plate, using trypticase soy broth (TSB) supplemented with 1% (w/v) glucose (Glu) as described previously [13] with modification. Bacteria were grown in TSB-1% Glu, in a shaker incubator (200 r.p.m.) at 37 ºC for 18 hours (hr.). The cultures were diluted 1: 100 inTSB-1% Glu and 200 µl was inoculated into each well, well contained only TSB broth serves as sterility control. The microtiter plate was incubated at 37ºC for 18 hr. Supernates were removed from each well and biofilms were gently washed twice with 0.85% NaCl, then dried and fixed at 65 ºC for 1 hr. Subsequently, the plates were stained with 0.1% (w/v) methylene blue for 10 min, gently washed twice and the quantitative analysis of biofilm production was performed by adding 200 µl of 95% ethanol for 10 min. Finally, 200µl from each well was transferred to a new microtiter plates and the absorbance of the methylene blue present in the destaining solution (ethanol) was measured at 580 nm by microplate reader.

Statistical analysis

The categorization of biofilm was performed based on Statistical analysis of at optical density (OD) 580nm by using Microsoft Excel 2010, with one-way analysis of variance (Duncans multiple range test). Differences were considered significant when α=0.05.

Result and discussion:

From 107 urine swabs, eighteen (5males and 13 females) specimen were identified as S.aureus and thirty were identified as S.epidermidis (7males and 23 females). The study showed that females had higher rate of UTI than males this maybe due to the anatomical difference between urethra of males

and females since the urethra in females, is much shorter and closer to the anus. As a woman's estrogen levels decrease with menopause, her risk of UTI increases due to the loss of protective vaginal flora [14]. Moreover, the intercourse leads to small wounds in the urethra and push bacteria into the bladder, Addition to the above; the older women are more vulnerable to UTI infection than younger woman as a result of hormonal changes and differences in acidity of the vagina [15 and 16]. Interestingly, the results showed that S.epidermidis are higher prevalence than S.aureus.

S.epidermidis often causes UTI in hospitalized patients, and sometimes their presence are associated with the use of urinary catheter, certain strains of S.epidermidis can produces slime layer which used it; to adhere on living and non living surfaces, to protect from host defenses and antibiotics and to help in infection each of the prostate and urethra in the older men [15 and 17]. In addition to other factors, such as environmental and geographic and genetic related to the lives of patients with urinary tract themselves [18].

Susceptibility test was done for all, 18 S. aureus and 30 S.epidermidis isolates using oxacillin disk diffusion method. The results revealed that the prevalence of MRSA and MRSE were 55.5% and 26.6%, respectively table ١. The biofilm forming ability was studied only for MRSA and MRSE, the statistical analysis of biofilm OD values for MRSA and MRSE revealed four groups Table٢.

Table 1- Prevalence of MRSA and MRSE in UTI

S.aureus (18 isolates)			**S.epidermidis (30 isolates)**		
MRSA		MSSA	MRSE		MSSE
10(55.5%)		8(44.5%)	8(26.6%)		22(73.4%)
female	male		female	Male	
9(90%)	1(10%)		6(75%)	2(25%)	

Table-2-Classification of MRSA and MRSE biofilm formation by microtiter plate method.

OD values at 580nm	**Biofilm category**
0.459 to 0.551	Weak
0.621 to 0.865	Mild
1.422 to 1.533	Strong
1.577 to 1.680	Highly-strong

MRSA isolates were confined between two groups, weak producers (7 out of 10) and mild producers (3 out of 10). While MRSE isolates were confined between strong producers (6 out of 8) and highly strong producers (2 out of 8). It is worth mentioning that the maximum biofilm of MRSA was A7 isolate (0.865nm). While the minimum was A8 isolate (0.459nm) in another hand the maximum biofilm formation of S.epidermidis was E6 isolate that showed strongest production (1.680nm), while the minimum biofilm producer was E52 isolate(1.310nm) (table٣).The fact that biofilm in MRSE are significantly higher than MRSA (figure1) may explain why S.epidermidis are more prevalence in UTI than S. aureus .Gould et al. (2010) stated that biofim ability increases the risk for UTIs and the risk of bacteriuria (bacteria in the urine) is between three to six percent per day and prophylactic antibiotics are not effective in decreasing symptomatic infections [19].

Table -٣- Biofilm formating capacity of MRSA and MRSE

	Isolate code	OD±SD	Biofilm category
MRSA (10 isolates)	A2	0.482±0.021656	weak
	A7	0.865±0.036056	mild
	A8	0.459±0.050954	weak
	A9	0.750±0.040266	mild
	A10	0.621±0.08903	mild
	A11	0.536±0.030172	weak
	A12	0.54٩±0.041621	weak
	A13	0.551±0.040649	weak
	A14	0.543±0.044959	weak
	A16	0.502±0.070685	weak
MRSE (8 isolates)	E6	1.680±0.020526	highly strong
	E7	1.533± 0.03166	strong
	E13	1.346±0.019502	strong
	E26	1.422±0.010017	strong
	E30	1.55±0.042579	strong
	E46	1.348±0.034646	strong
	E52	1.310±0.094694	strong
	E53	1.577±0.038501	highly strong

- Each datum is a mean of triplicate and subtracted from (0.158) (control OD value). LSR=0.1235.

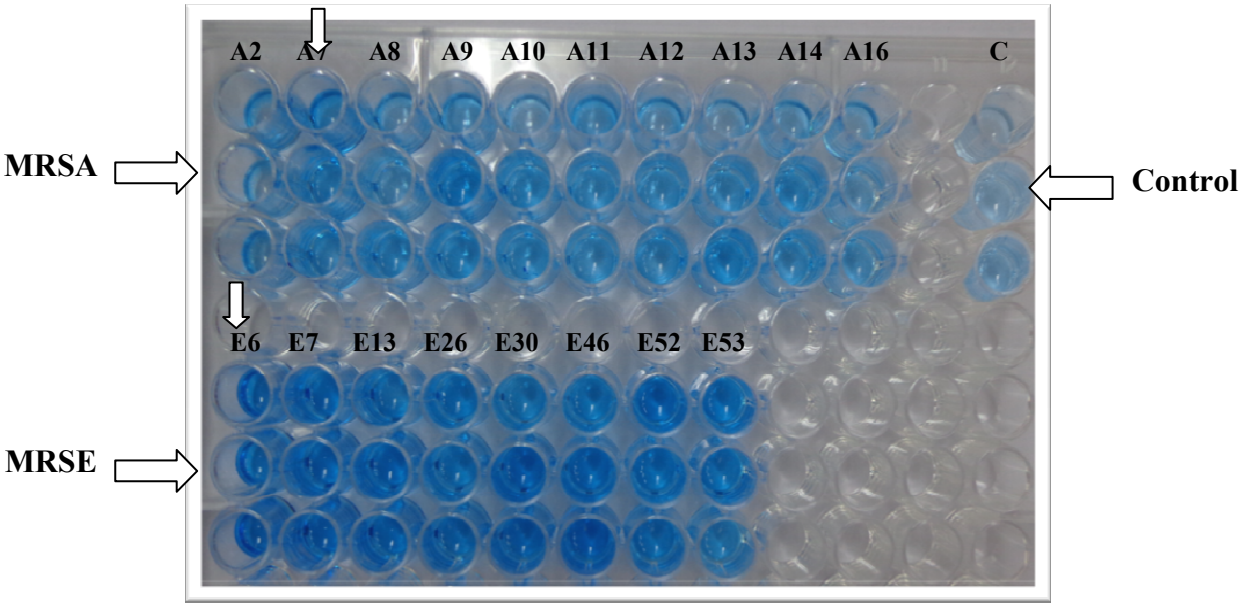

Figure -1- MRSA and MRSE biofilm with microtiter plate after 18hr of incubation.

- 7A=maximum biofilm formation of MRSA.
- 6E= maximum biofilm formation of MRSE.

The biofilm-producing MRSE isolates detected carried the icaADBC operon (intercellular cluster adhesin) involved in the production of the polysaccharide intercellular adhesin (PIA) that is functionally necessary for cell-to-cell adhesion and biofilm accumulation. The accumulation-associated protein (AAP) as important genetic determinants of slime production and AtlE was suggested to play a role in the attachment to polystyrene surfaces and to vitronectin, thereby contributing to biofilm formation of MRSE on implanted

polymers [20,21 and 22]. It is important to remark that all MRSE isolates displaying the strong biofilm producer phenotype carried all the three genes, in addition to contain MRSE unusually high amounts of teichoic acids that give very strong biofilm [23 and 24]. This explains why MRSE has higher ability to produce biofilm
than MRSA. Previous studies showed that biofilm infections are a major medical problem, with,with S.aureus and coagulase negative staphylococci, manily S.epidermidis, as the leading species responsible for chronic polymer-associated infections [25 and 26]. The ability to adhere to materials and promote formation of a biofilm is an important feature of the pathogenicity of bacteria involved in foreign body infections. Foreign body-related infections (FBRIs), particularly catheter-related infections, significantly contribute to the increasing problem of nosocomial infections staphylococci account for the majority of FBRIs [27].

References:

1. Shareef,S.A. and Yagoub,S.O. **2006** . Incidence and isolation of bacteria associated with nosocomail urinary tract infection (UTI) in Sudanese women. *Research Journal of Microbiology*. 1(6), pp:534-539.
2. Centers for Disease Control and Prevention. **2004**. The Burden of Chronic Diseases and Their Risk Factors: National and State Perspectives. 4, pp:126-7.
3. Collier,L., Balows,A. and Sussman,M. 1**998**. Urinary tract infection. Topley and Wilsons Microbiology and Microbial Infection.Urinary Tract Infection.6 Edn, vol.3 Edward Annold, London.
4. Akinjogunla, O. J.; Odeyemi, A. T. and Olasehinde, G. I.**2010**. Epidemiological Studies of Urinary Tract Infection (UTI) among Post-menopausal Women in Uyo. Metropolis, SouthSouth, Nigeria. *Journal of American Science*.6 (12).
5. Saising, J., Singdam, S., Ongsakul, M. and Voravuthikunchai, S.P. **2012**. Lipase, protease, and biofilm as the major virulence factors in staphylococci isolated from acne lesions. BioScience Trends. 6(4), pp:160-164.
6. Oleksy, A., Golonka, E., Bańbula, A.,Szmyd, G., Moon, J. , Kubica , M., Greenbaum, D., Bogyo, M., Foster, T. J.; Travis, J. and Potempa, J. **2004**. Growth phase-dependent production of a cell wall-associated elastinolytic cysteine proteinase by Staphylococcus epidermidis. Biol. Chem. 385, pp:525–535.
7. Walencka, E., Sadowska, B., Różalska, S.,Hryniewicz,W.,Różalska,B.**2006**.Staphyl-ococcus aureus biofilm as a target for single or repeated doses of oxacillin, vancomycin, linezolid and/or lysostaphin. Folia Microbiol.51,pp:381-386.
8. Martineau,F.; Picard, F.J.; Lansac,N.; Menard,C.; ROY, P.H.; Ouellette, M. and G. Bergeron,M.G. **2000**.Correlation between the Resistance Genotype Determined by Multiplex PCR Assays and the Antibiotic Susceptibility Patterns of Staphylococcus aureus and Staphylococcus epidermidis. Antimicrobial Agent and Chemotherapy. 44(2), pp:231–238.
9. Götz, F., Bannerman, T. and Scheifer, K. **2006**. The genera Staphylococcus and Micrococcus. In: Dworkin, M., Failkow, S., Roseberg, E., Schleifer, K.,Stackebrant, E. The Prokaryotes handbook on the biology of bacteria. Springer. New York. USA. 4, pp:5-75.
10. Talan, D.A.,Staatz, D., Staatz, A. and Over turf, G.D. **1989**. Frequency of Staphylococcus intermedius as human nasopharyngeal flora. *Journal of Clin. Microbiol*. 27, pp:23-93.
11. De, N. and Godlove, M. **2010**. Prevalence of S. aureus and S. epidermidis among patients with indwelling catheters and their antibiogram using some commonly used antibiotics. *Journal of American Science*. 6**(**11), pp:515-520.
12. Clinical Laboratory Standards Institute. (CLSI). **2006**. Performance standards for antimicrobial susceptibility testing. Twentieth informational supplement. CLSI document M100-S20. Wayne, PA.
13. Amaral, M. M., Coelho, L. R.F, lores, R. P., Souza, R. R., Silva- Carvalho, M. C., Teixeira, L. A., Ferreira-Carvalho, B. T. and Figueiredo, A. M. S. **2005**. The predominant variant of the Brazilian epidemic clonal complex of methicillin-resistant Staphylococcus aureus has an enhanced ability to produce biofilm and to adhere to and invade airway epithelial cells. *Journal of Infect Dis.*192, pp:801–810.
14. Dielubanza, E.J. and Schaeffer, A.J. **2011**. Urinary tract infections in women. The

Medical clinics of North America. 95 (1), pp:27–41.

15. Jaloot, A.S., AL-hadithi,H.A. and AL-heeti,N.H. **2007**. Astudy on urinary tract infections in al ramadi general hospital in al anbar governoratwe. *Anbar Journal of Science*.**1** (3).

16. Rahman, M.A., Talukder, S.I., Khatoon, M.R. and Arman, R. **2010**. Urinary Tract Infection in Pregnancy: a Clinical Problem. *Dinajpur Med Col Journal*. **3** (2), pp:59-62.

17. Shanson, D.C.**2000**. Microbiology in Clinical Practice. 3rd ed. Butterworth Heinemann .Oxford. p:326 – 339.

18. AL-Fahdawi, AR.M.G. **2001**. Influence of blood groups on the availability of receptors of uroepithelial cells for attachment of uropathogenic bacteria causing urinary tract infections (UTIS). M.Sc. thesis college of Med. University of Baghdad .Iraq.

19. Gould, C.V., Umscheid, C.A., Agarwal, R.K., Kuntz, G. and Pegues DA. **2010**. "Guideline for prevention of catheter-associated urinary tract infections 2009". Infect Control Hosp Epidemiol .31 (4), pp:319–26.

20. Vandecasteele, S.J., Peetermans, W. E., Merckx, R. R., Rijnders,.B.J. and Van eldere,J. **2003**. Reliability of the ica, aap and atlE genes in the discrimination between invasive, colonizing and contaminant Staphylococcus epidermidis isolates in the diagnosis of catheter-related infections. Clin microbial infect. 9(2), pp:114-119.

21. Russell,W. ;and Herwald, H(eds). **2005**. Concepts in bacterial virulence. Contrib Microbiol. Basel, Karger. 12, p:1-27.

22. Yazdani, R., Oshaghi, M., Havayi, A., Pishva, E., Salehi, R. and Sadeghizadeh, M. **2006**. Detection of icaAD gene and biofilm formation in Staphylococcus aureus isolates from wound infections. *Iran Journal of Publ Health*. 35,pp:25-28.

23. De Araujo, G. L., Coelho, L. R., de Carvalho, C. B., Maciel, R. M., Coronado, A. Z., Rozenbaum, R., Ferreira-Carvalho, B. T., Sá Figueiredo, A. M. and Teixeira, L. A. **2006**. Commensal isolates of methicillin-resistant Staphylococcus epidermidis are also well equipped to produce biofilm on polystyrene surfaces. *Journal of Antimicrob. Chemother*. 57(5), pp:855-864.

24. Otto, M. 2004 .Virulence factors of the coagulase-negative staphylococci. Front. Biosci. 9, pp:841–863.

25. Gotz, F. 2002. Staphylococcus and biofilms. Mol Microbiol. 43, pp:1367-1378.

26. Ruzicka, F., Hola, V., Votava, M., Tejkalova, R., Horvat, R., Heroldova, M. and Woznicova, V.2004.Biofilm detection and the clinical significance of Staphylococcus epidermidis isolates .Folia Microbiol.49, pp:75-78.

27. Von Eiff, C., Jansen, B., Kohnen, W. and Becker, K. 2005. Infections associated with medical devices: pathogenesis, management and prophylaxis.Drugs.65, pp:179–214.